Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

# THE FATE OF FOREIGN ERYTHROCYTES INTRODUCED INTO THE BLOOD STREAM OF THE RABBIT *

William E. Cary

*From the Department of Hygiene and Bacteriology of the University of Chicago*

Altho it has been definitely established that the introduction of foreign red blood corpuscles into an animal produces profound physiologic changes, as evidenced by the appearance of specific antibodies in the body fluids, the actual fate of the cells thus introduced has not been determined. From the point of view of general biology, as well as from that of the particular field of immunity, however, the actual fate of cells so introduced appears to be of much importance in that the site and mode of the destruction of the foreign cells undoubtedly bear a direct relation to the site of antibody production, concerning which but few data have been secured.

It is the purpose of this paper to contribute evidence that foreign blood cells introduced into the circulating blood stream are engaged by and destroyed within certain fixed tissue cells of the liver and spleen. In this connection I wish to express my thanks to Professor P. Kyes for suggestions and criticism relative to this work.

Kyes[1] has recently shown, in a wide range of animals, that the physiologic destruction of the animal's own red blood corpuscles is accomplished by specialized, fixed tissue phagocytes, which he designates as hemophages. The hemophages are confined largely to the liver and spleen and are of the same type in both these organs. In different animal species, the blood destruction accomplished by the hemophages may be carried on predominately, or even exclusively, in the one organ or the other, the site of the destruction, however, being constant for a given species under normal conditions. Thus in birds, amphibia, and lower mammals, the hemophages are most active in the liver, whereas, in the rodents and other high mammals, the hemophage activity is most marked within the spleen. Kyes has further shown that the destruction of the red blood corpuscles by the hemophages under normal conditions is attended with a freeing of the

---

* Received for publication June 22, 1915.

[1] Internat. Monatschr. f. Anat. u. Physiol., 1914, 31, p. 543.

iron of the contained hemoglobin to such an extent that it is available for Perl's Prussian blue reaction in fixed tissues and affords a histologic method for the sharp differentiation of all active hemophages.

This demonstration of an actual intracellular destruction of red blood corpuscles within specialized cells under normal conditions, suggested the possibility that the same mechanism might operate in the destruction of foreign corpuscles introduced into the blood stream, and, further, that proof of this operation might be found in the increased activity of hemophages stimulated by the presence of a large number of foreign corpuscles. This increased activity should appear in the increased number of hemophages containing red blood corpuscles and displaying an iron reaction by virtue of the end products of the intracellular hemoglobin digestion.

To test the possibility of obtaining such proof, I have injected a series of twenty-six rabbits with beef corpuscles and compared the resulting hemophage activity in these animals with that of normal rabbits. Rabbits were selected as recipients, since, as Kyes has shown, in these animals, in contrast to many others, the liver does not contain active hemophages under normal conditions, the destruction of erythrocytes being accomplished by the splenic hemophages. Because of the known action of hemophages in the liver of other species, however, and the action of these cells under certain pathologic conditions, it was considered possible that, under the demand for a great increase in the destruction of erythrocytes, the liver even in rabbits would act as a supplementary organ in this function, and such a participation would contrast sharply with the normal condition. The red blood corpuscles injected were in all instances serum-free, being prepared by multiple washings in the isotonic salt solution. The concentration of corpuscles in the suspension actually employed for injection was in all instances equivalent to that of the blood as drawn. The unit for each injection was 5 c.c. of such a suspension and the avenue of introduction was the lateral ear vein. In the preparation of the tissues for histologic study, the technic employed was the same as that given by Kyes. This is an application of Perl's test for iron to fixed tissues in combination with suitable counter-stains. In detail, the method is as follows:

Fix thin slices of tissue for eighteen to twenty-four hours in Müller's fluid plus 5 percent mercuric sublimate. Imbed in paraffin and section to 4 microns. Fix sections to slide and stain five to ten minutes with acid carmin. Wash, and transfer to equal parts of a 2 percent aqueous solution of potassium ferrocyanid and a 2 percent aqueous solution of hydrochloric acid, the combined solution containing also 2 percent of sodium chlorid. Remove after three to ten minutes, wash in distilled water, and pass quickly through a 1.0 percent aqueous eosin solution. Dehydrate in alcohol, clear in xyol, and mount in Canada balsam.

With this method, there are constantly to be found in the spleen of normal rabbits a large number of hemophages which are characteristically differentiated by virtue of a content of Prussian blue, the presence of which results from a partial liberation of iron from ingested erythrocytes. In the liver, on the other hand, no such differentiation of hemophages occurs. The differentiated cells of the spleen are similar to those described by Kyes in detail in the spleen of pigeons. Within a majority of such cells are to be observed erythrocytes in various stages of digestion. The number of hemophages active in the spleen at a given time varies somewhat for different individuals, even under normal conditions. But this variation is not marked and the tissues from a series of six normal rabbits serve as a basis of comparison in the following observations and are designated as Group I.

The rabbits injected with foreign corpuscles fall into four additional groups, according to the amount of hemophage activity displayed in the spleen and liver. Two factors were varied in the experiments, namely, the number of injections given to a single animal and the length of the period between the last injection and the killing of the animal. The results of the experiments, in general terms, show that the injection of foreign red blood corpuscles does increase the activity of the hemophages markedly. This activity, moreover, varies directly with the number of erythrocytes injected and is displayed in an increase of the number of hemophages containing these cells and the products of their digestion. Altho the great increase in the hemophage activity is apparent in the spleen, the clearest evidence as to this increase is to be seen in the liver, where the experimentation results in the presence of very great numbers of active hemophages in contrast to the total absence of such cells under normal conditions. As a basis for the discussion of the results in detail, Table 1 is introduced.

This table displays the relative number of hemophages observed in the spleens and livers of the total series of thirty-two rabbits. The series is divided into five groups. The six rabbits of Group I were normal controls and serve as a basis of comparison. It is to be noticed that active hemophages were constantly present in the spleens, but never in the livers of these normal rabbits. A very few active hemophages occur in the bone marrow also, but inconstantly, and the number is but slightly, if at all, modified by the experiment described.

In all the animals of Group II, the only distinctive result was a decided increase in the number of active hemophages in the spleen. No change appeared in the liver.

TABLE 1

| Rabbit | Number of Injections* and Time Elapsing After Last Before Rabbit was Killed | Presence of Active Hemophages | |
|---|---|---|---|
| | | in Liver | in Spleen |
| | Group I—Controls | | |
| 1 | Normal | — | $+$ |
| 2 | Normal | — | $+$ |
| 3 | Normal | — | $+$ |
| 4 | Normal | — | $+$ |
| 5 | Normal | — | $+^{2}$ |
| 6 | Normal | — | $+$ |
| | Group II—One Injection | | |
| 1 | Sixteen hours | — | $+^{3}$ |
| 2 | Twenty-four hours | — | $+^{3}$ |
| 3 | Twenty-four hours | — | $+^{3}$ |
| 4 | Ninety-six hours | — | $+^{2}$ |
| 5 | Ninety-six hours | — | $+^{3}$ |
| 6 | Ninety-six hours | — | $+^{4}$ |
| 7 | Ninety-six hours | — | $+^{2}$ |
| 8 | One hundred and twelve hours | — | $+^{14}$ |
| | Group III—One injection | | |
| 1 | Twenty-two hours | $+$ | $+$ |
| 2 | Ninety-six hours | $+^{2}$ | $+^{8}$ |
| 3 | Ninety-six hours | $+$ | $+^{10}$ |
| 4 | Ninety-six hours | $+$ | $+^{6}$ |
| | Group IV—Three injections, successive days | | |
| 1 | Twenty-four hours | $+$ | $+$ |
| 2 | Twenty-four hours | $+^{2}$ | $+^{10}$ |
| 3 | Twenty-four hours | $+^{2}$ | $+^{9}$ |
| 4 | Twenty-four hours | $+^{2}$ | $+^{10}$ |
| 5 | Twenty-four hours | $+^{2}$ | $+^{8}$ |
| 6 | Twenty-four hours | $+^{2}$ | $+^{7}$ |
| 7 | Twenty-four hours | $+^{2}$ | $+^{5}$ |
| 8 | Twenty-four hours | $+^{4}$ | $+^{5}$ |
| 9 | Forty-eight hours | $+^{2}$ | $+^{8}$ |
| 10 | Forty-eight hours | $+^{3}$ | $+^{15}$ |
| 11 | Forty-eight hours | $+^{5}$ | $+^{10}$ |
| 12 | Forty-eight hours | $+^{2}$ | $+^{10}$ |
| | Group V | | |
| 1 | Four injections, alternate days. Twenty-two hours | $+^{2}$ | $+^{10}$ |
| 2 | Eleven injections, alternate days. One hour | $+^{5}$ | $+^{15}$ |

* Each injection was the introduction intravenously of 5 c.c. of a suspension of washed beef erythrocytes in a concentration equivalent to that of the blood as shed.

The results in Group III differ from those in Group II in that, in addition to a marked increase in the hemophages of the spleen, there appear also, for the first time, active hemophages in the liver. In the instance of Rabbit 1, the active hemophages were found in the liver twenty-two hours after a single injection, this being the earliest occurrence noted.

The twelve animals included in Group IV received, in contrast to those of the preceding two groups, a multiple number of injections, namely, three. Eight of these animals were injected on successive days and killed twenty-four hours after the last injection. The other four animals were similarly injected but were killed only after a lapse of forty-eight hours. All these animals displayed a very distinct increase in the active hemophages in the spleen and the abnormal presence of large numbers of active hemophages in the liver. In general, the rabbits of this group showed a distinctly greater hemophage activity, both in the liver and in the spleen, than that presented by rabbits of Groups II and III, which received but a single injection of corpuscles.

The two rabbits of Group V received a greater number of injections than any of the other animals. ,The former received four, and the latter eleven injections. In both instances, there was an extensive increase in the number of the active hemophages in the spleen and the presence of great numbers of similar cells in the liver. The results in the bone marrow were not definite.

From these results it is seen that the injection of foreign red blood corpuscles profoundly affects the hemophage activity of the recipient. Not only are the injected cells eliminated by an increase of active hemophages in the spleen, but also by an active participation of hemophages in the liver, whereas no such participation occurs in this organ normally in this species. It is to be observed also that, within limits, the degree of hemophage activity varies with the extent of the injection of foreign cells.

With the determination of the phagocytic activity of hemophages in the spleen and liver in the destruction of foreign erythrocytes, recurs the question referred to at the commencement of this paper: Do the cells which destroy foreign erythrocytes participate in the formation of their antibodies? If so, the liver and the spleen in animals injected with erythrocytes might well be expected to show a high concentration of antibodies to those cells. In a subsequent paper, I shall show that this is the case.